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COMPARATIVE STUDY OF *WOODFORDIA FRUTICOSA* AND *CATHARANTHUS ROSEUS* LEAVES FOR PHYTOCHEMICAL, ANTIOXIDANT AND ANTIBACTERIAL PROPERTIES

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ABSTRACT

In present study various solvent extracts of leaves have been used for comparative study of Woodfordia fruticosa and Catharanthus roseusn for their natural bioactive compound, antioxidant properties and antibacterial properties and were confirmed by TLC. Here Chloroform extracts was found to be effective solvents for alkaloid and terpenoid, Methanol was for flavonoid and tannin and aqueous extract was found to be effective solvent for cardiac glycoside and saponin. The results also revealed that Woodfordia fruticosa leaves extracts has higher percentage of tannin (1.496%) as compare to Catharanthus roseus with value (0.693%) whereas antioxidant property of Woodfordia fruticosa was poor (1.1 µmole/ml glutathione) in comparison with Catharanthus roseus (25.09 µmole/ml glutathione). Antimicrobial assay showed that methanolic extracts of Woodfordia fruticosa in comparison with Catharanthus roseus were more effective than other solvent extracts with (14mm/9mm, 19mm/4mm, 17mm/8 mm, 11mm/9mm) zone of inhibition against Bacillus subtilis, Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa respectively. This comparative results obtained in the present study would be useful option for future drugs discoveries to cure various diseases.

KEYWORDS: Antioxidant, Catharanthus roseus, Medicinal Plants, Phytochemical, Secondary Metabolites & Woodfordia fruticosa

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Original Article

INTRODUCTION

Natural products are secondary metabolites which are derived from herb or animal source and used in drug discovery and drug design. Medicinal plants have been the mainstay of traditional herbal medicine amongst rural dwellers worldwide since antiquity to date (Yalavarthy and Thiruvengadarajan, 2013). Like other forms of alternative therapy, herbal medicine attempts not to cure disease, but helps the body to return itself in the state of balance *i.e.* health. In the present scenario of speedy and hectic life style, mental anxiety, low physical movement, many diseases and disorders are mounting very rapidly. A bulky number of bioactive compounds hold by herb that can help to alter the body's chemistry to come back in their normal health state (Shrivastava, 2009). Infectious diseases also are the world's leading cause of premature death and caused by variety of bacteriological agents including *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa* which is most common (Parmar and Rawat, 2012; Bhattarai and Bhuju, 2011). Now a day, Due to the nonstop use of antibiotics human pathogenic bacterium has been showed resistance to many drugs and antibiotics also have adverse effects

on host, demand to develop alternative antimicrobial drugs for the treatment of infectious disease (Manandhar, 2002). A large number of people in developing world about 3.4 billion people (88% of world inhabitants) who rely mainly on conventional remedy for their crucial health care (Doughari *et al*., 2009; Yadav and Dixit, 2003). According to world health organization, a medicinal plant is any plant which, in one of its organ contain substance that can be used for therapeutic purposes or which are precursor of chemopharmaceuticals semi synthesis. The limiting amount of drugs and newly arising disease, boost up the medicinal plant research to develop new pharmacological compound (Cordell, 1993).

Woodfordia fruticosa/Dhawai is spreading evergreen shrub belongs to the family lythraceae. It is native of Asia and Africa and it is widely distributed in throughout the world (Thakur *et al*, 1989). Various natural compounds like tannins, phenols, terpenoids/steroids, carbohydrates, etc. have been present in this plant. By tradition, its entire part is valuable, but it is commonly cultivated for its leaves and flowers. It is very useful in the case of Fever, Dysentery, Headache, Diarrhea, Ulcer and wound by playing acting role of stimulant, astringent and Tonic (Grover and Patni, 2013; Chadha, 1976). *Catharanthus roseus*/Sadabahar is a semi woody evergreen perennial herb, usually grown as an annual in the flower bed of Apocynaceae family. It is native to the Caribbean Basin and has historically been used to treat a wide assortment of diseases (Nayak and Pinto Pereira, 2006). The whole plants are very useful for different purposes, but the leaves and root are generally used. Roots and leaves of this plant contain more than 100 alkaloids. Traditionally, *C. roseus* organic extracts is used for the treatment of many disease *viz.* malaria, diabetes,sore throat, eye irritation etc. It is also used as an astringent and antioxidant which acts like a radical scavengers for protection of human against free radicals by inhibiting the oxidizing chain reactions (Patharajan, and BalaAbirami, 2014).

In the present study we have selected *Woodfordia fruticosa* medicinal plants for the estimation and screening of secondary metabolite, antibacterial activity in the different solvent extracts of leaves and compared the activity of active constituent with known important medicinal plant *Catharanthus roseus* in order to search for an alternative option of herbal medicine in pharmacological research.

MATERIALS AND METHODS

Well developed plant of *Woodfordia fruticosa* and *Catharanthus roseus* collected from Garden of Universal Medicaments Pvt. Ltd, Shanti Nagar, Nagpur (M.H.). Leaves of these plants were dried in sun light for 3 days. The present study was conducted at Unijules life science Pvt. Ltd. Nagpur (M.H).

Methods

Extraction of Secondary Metabolite from *Woodfordia fruticosa* and *Catharanthus roseus* Leaves Extracts

For the extraction of secondary metabolites 5 gm dried leaves of *Woodfordia fruticosa* and *Catharanthus roseus was* taken in soxhlet's extractor and heated repeatedly for 18 hrs with Hexane (200mL x 2) and subsequently with Chloroform, Methanol and Distilled water (200mL x 2) [Precaution should be taken, that continuous flow of water is maintained].

Biochemical Test for Screening of Secondary Metabolites

Secondary metabolites present in *Woodfordia fruticosa* and *Catharanthus roseus* leaves extracts were phytochemical screened by above four solvent using following biochemical test as suggested by Harborne (1998) and Parekh and Chanda (2007).

Test for Alkaloids

On boiling water bath 1ml of extract of all solvent used were heated with 2N HCL (5mL) and filter the mixture after cooling. Further filtrate was treated by little drops of Wagner's reagent for turbidity or flocculation because amount of turbidity obtained is directly proportional to the quantity of alkaloid presented in the extract/sample (Salehi and Surmaghi *et al.,* 1992).

Test for Cardiac Glycosides

Glacial acetic acid (0.2ml) containing 1drop of $FeCl_3$ (0.5ml) was used to treat 0.5 ml of extract. After that 1 ml of H_2SO_4 was layered, which forms a brown ring at the interface, indicates the presence of cardiac glycosides. Here the size of the ring directly proportionate the amount of cardiac glycosides present in the extracts (Ajaiyeobu, 2002) .

Test for Flavonoid

Test of flavonoid started by taking 5ml of dilute ammonia was added to the portion of filterate followed by adding up concentrated H_2SO_4. Yellow color observed in the solvent extracts indicated the presence of flavonoid in solution and the degree of yellow coloration determines the amount of flavonoid present in extracts (Sofowara, 1993).

Test for Saponins

The extracts of different solvents were dissolved in water and mix well by shaking. Formation of froth during shaking indicates the presence of saponins. The degree of froth formed determines the quantity of Saponins present in extracts (Kapoor et al., 1969).

Test for Tannins

Presence of tannin in the extracts done by taking 5ml of extract in a test tube and added few drops of 0.1% $FeCl_3$. A brownish green/bluish black color precipitation indicates the presence of tannins. The quantity of precipitation formed proportionate to the amount Saponins (Segelman *et al*., 1969).

Test for Terpenoids

In a test tube 5ml of solvent extracts was taken and mixed with 2ml of chloroform and 3ml of concentrated H_2SO_4 invidually. At the interface a reddish brown color indicates the presence of terpenoids in the extracts (Evans, 1997).

Confirmation of Secondary Metabolites by TLC

The confirmatory test for the above secondary metaboliteswas done by using thin layer chromatography (TLC) as reported by (Waksmundzka-Hajnos *et* al., 2008 and Harborne, 1998) with effective solvent extracts of each metabolites identified by biochemical test. Information of mobile phase and spraying reagents used are given in **table 2**.

Quantification of Tannin in *Woodfordia fruticosa* and *Catharanthus roseus* Leaves

The amount of tannin presents in *Woodfordia fruticosa* and *Catharanthus roseus* Leaves were quantified with Lowenthal Permanganate titration method given by Lowenthal (1877) and Rajpal (2005). For this 1 gm of sample was dissolved in the 100 ml H_2O, further filter and transfer the 10 ml of filterate to a conical flask. Further 750 ml of H_2O and indigo sulphonic acid solution (25 ml) was added and titrated with constant stirring against N/10 KMnO4 till golden

yellow color observed. A blank test was also done simultaneously by titrating with 25 ml of indigo sulphonic acid in 750 ml of water.

Here each ml of N/10 KMnO4 = 0.004157 gm of Tannins.

Estimation of Glutathione in *Woodfordia fruticosa* and *Catharanthus roseus* Leaves by Ellman Assay

The antioxidant property of *Woodfordia fruticosa* and *Catharanthus roseus* was determined by the protocol as given by Eyer and Podhradsky (1986) and Rotruck et al., 1973) in triplicates by using following steps:

1 ml of sample was incubated for 90 seconds with 0.8 ml of 0.4 M sodium phosphate buffer (pH 7.0), 0.2 ml of 10 mM sodium azide (65 mg/100 ml water), 0.4 ml of 4 mM reduced glutathione (GSH), 0.2 ml of 2.5 mM H_2O_2 and 0.4 ml of water. The reaction was completed with 1 ml of 10% trichloroacetic acid (TCA) and later than centrifugation at 3000 rpm for 10 minutes. After that 3 ml of phosphate buffer and 1ml of DTNB (5,5′-dithiobis-(2-nitrobenzoic acid) reagent (0.04% DTNB in 1% sodium citrate) in the 2 ml of the supernatant collected after centrifugation. The color changes identified was read at 412 nm in the Systronics make spectrophotometer and the enzyme activity is articulated in terms of µM/mg. This enzyme assay was done 4 times and the outcome was presented in the form of mean value.

Here total of TNB (2-nitro-5-thiobenzoate) formed= quantity of Glutathione in leaf extract

Antibacterial Assay of *Woodfordia fruticosa* and *Catharanthus roseus* Leaves Extracts

For the antibacterial assay of different solvent extracts of *Woodfordia fruticosa* and *Catharanthus roseus* leaves different bacterial strains *viz.* Gram-positive *Staphylococcus aureus*, *Bacillus subtilis* and Gram-negative *Escherichia coli*, *Pseudomonas aeruginosa* were used. Microbial strains for the current study were procured from Unijules Life Sciences, Laboratory, Nagpur, India. The bacterial culture were grow and maintained on nutrient agar (Hi Media, India) slope at 4ºC and sub culturing is used for the activation purposes.

Antibacterial Assay

For the antibacterial property of different solvent extracts was done by disc diffusion method given by Taylor *et al*., (1995). Mueller Hinton agar no. 2 medium was primed in each sterilized petri plates of 9 cm diameter and permitted to solidify. Bacterial suspension culture was primed aseptically by dH_2O) under laminar air flow. The spread plate technique was used to cultured microbial suspension (100- 150 µl). The disc diffusion assay method was used to measure zone of inhibition after 24 hrs. extracts free pure solvent was used as a control thorough out the experiment. The control zones of inhibition were subtracted from the test zones of inhibition for final calculation. The experiments were performed in replicated manner and presented as average values.

Results

In the current study extraction, screening, TLC, antioxidant test, quantification of tannin, and antibacterial assay of leaf extracts of *Woodfordia fruticosa* and *Catharanthus roseus* have been done in comparative manner. The results obtained are as followed:

Different solvent Extracts of *Woodfordia fruticosa* (W) and *Catharanthus roseus* and their VISUAL Interpretation

Visually the various solvent extracts of *Woodfordia fruticosa* and *Catharanthus roseus* leaf revealed that methanol extracts has highest concentration of crude extracts followed by distilled water extracts of leaves whereas hexane

extracts has lowest concentration in both plants (figure 1).

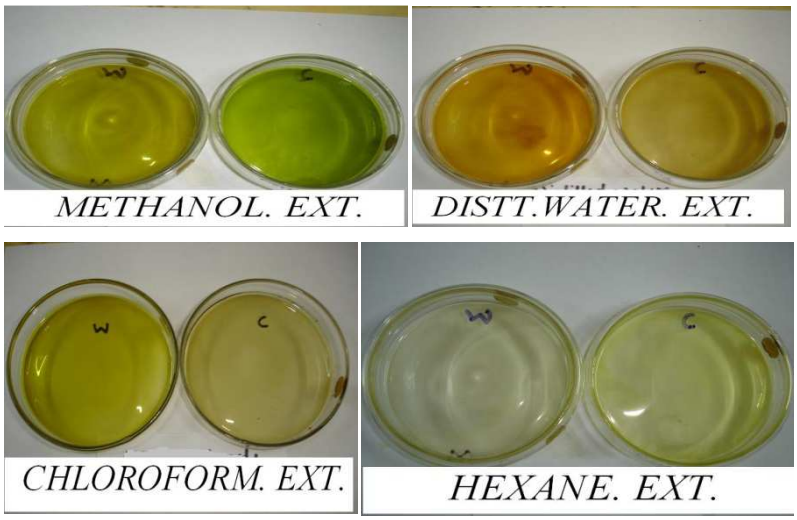

Figure 1: Different Solvents Extracts of *Woodfordia fruticosa* (W) and *Catharanthus roseus* (C) Leaves

Phytochemical Screening of Secondary Metabolite

While screening the secondary metabolites in various solvent extracts biochemically it was observed that *Woodfordia fruticosa* and *Catharanthus roseus* both plant leaves extracts gave present or absent type results conformational changes (table 1). The solvent extracts which showed the higher amount of conformational changes called as effective solvents for particular metabolites and would be further used for confirmation by TLC. *Catharanthus roseus* leaf extracts had higher amount of alkaloid, flavonoid and terpenoids while *Woodfordia fruticosa* had higher amount cardiac glycosides, Saponins and tannin. The present study also showed that chloroform is an effective solvent for alkaloid and terpenoids extraction, Methanol is an effective solvent for flavonoid and tannin extraction and water is an effective solvent for cardiac glycosides and saponins extraction from *Woodfordia fruticosa* and *Catharanthus roseus* leaves. Here hexane extracts was not identified as effective solvents for any of these metabolites **(Table 1)**.

Table 1: Screening of Secondary Metabolites in *Woodfordia fruticosa* and *Catharanthus roseus*

Secondary Metabolites/Extracts	Conformational Changes	Chloroform		Methanol		Aqueous		Hexane		Effective Solvent
		W	C	W	C	W	C	W	C	
Alkaloid	Yellow with turbidity	++	+++	+	+	-	+	-	+	Chloroform
Cardiac glycosides	Brown ring	-	-	++	+	++	+	-	+	Aqueous
Flavanoids	Yellowness	+	-	+	+++	+	+	-	-	Methanol
Saponins	Foam	+	+	-	-	++	+	-	-	Aqueous
Tannin	Blue black Precipitation	++	+	+++	++	+	+	+	+	Methanol
Terpenoids	Brown ring	++	+++	+	-	-	+	-	-	Chloroform

Where, W = *Woodfordia fruticosa*; C = *Catharanthus roseus*; +++ = present in higher concentration; ++ = present in medium concentration; + = present in lower concentration; dash (-) = absent

TLC Results of *Woodfordia Fruticosa* and *Catharanthus roseus*

The confirmatory results of TLC for the presence of secondary metabolites (Alkaloids, Tannins, Terpenoids, Flavonoid and Saponins) in both the plants leaves extracts have been done in their respective effective solvent. The Rf

value was found ~ same in both the plants for the particular metabolites as compare to standard as illustrate in **table 2**.

Table 2: TLC Confirmation of Secondary Metabolites Presents in *Woodfordia fruticosa* and *Catharanthus roseus* Leaves Extracts with Different Mobile Phase and Spraying Agents

Secondary Metabolites	Effective Solvent Extracts	Mobile Phase	Spraying Reagents	No. of Spots	Spot Color	RF Value
Alkaloids	chloroform	Butanol: Glacial Acetic acid: Water (10:10:5)	Zink chloride	1	pink	0.9500 (W) 0.9571 (C)
Cardiac glycosides	Distilled water	(chloroform: methanol: water (80:19:1)	Chloramine-trichloroacetic acid	1	Yellowish brown	0.1000 (W) 0.1021 (C)
Flavanoids	methanol	n-Butanol: Acetic acid: Water (6:1:2)	Bromocresol Green	1	Light green	0.9482 (W) 0.9396 (C)
Saponins	Distilled water	Chloroform: methanol: Water (6.5:3.5:1.0)	Anisaldehyde, glacial acetic acid and conc. H_2So_4	1	yellow	0.1338 (W) 0.1417 (C)
Tannins	methanol	Butanol: Acetic Acid: water (6:2:2)	Ferric chloride	1	black	0.9500 (W) 0.9583 (C)
Terpenoids	chloroform	Toluene: Ethyl acetate: Water (8.5:1.5)	Anisaldehyde - Sulphuric Acid	1	brown	0.9615 (W) 0.9711 (C)

Glutathione Assay

The value obtained in the antioxidant test of *Woodfordia fruticosa* and *Catharanthus roseus* leaves extracts by Glutathione assay tells that amount of glutathione is higher in *Catharanthus roseus* leaf extracts with value of 25.09 µmole/ml as compare to *Woodfordia fruticosa* leaves extracts with value 1.14 µmole/ml. So the antioxidant property of *Catharanthus roseus* is higher than *Woodfordia fruticosa*. The anti oxidant property of the both sample are positive; steps for extracting and also increasing the glutathione content from these sample should be developed.

Quantification of Tannin

The quantification of tannin showed that tannin percentage (w/w of tannic acid) in 1 gm sample of *Woodfordia fruticosa* leaf was higher (1.496 %) as compare to *Catharanthus roseus* (0.693 %). The Tannins fraction have high medical activities thus steps need to be developed to increase the tannin content of the plant and also good extraction steps for extracting tannins need to be developed.

Antibacterial Assay

The antibacterial activity of *Woodfordia fruticosa* and *Catharanthus roseus* leaf extracts from different solvent showed that out of four solvent leaves extracts methanol, chloroform and aqueous extracts of *Woodfordia fruticosa* and *Catharanthus roseus* showed the zone of inhibition against four bacterial strains whereas hexane extracts of both plants showed no zone of inhibition against these microorganism. In all the solvents extracts *Woodfordia fruticosa* leaves extracts showed higher zone of inhibition than *Catharanthus roseus* leaf extracts against studied microbes (**table 3**).

Table 3: Antibacterial Assay of Different Extracts of *Woodfordia fruticosa* and *Catharanthus roseus*

Microbial Strains	Diameter of Clear Zone (mm)							
	Hexane Extracts		Chloroform Extracts		Methanol Extracts		Aqueous Extracts	
	W	C	W	C	W	C	W	C
B.subtilis	-	-	13	7	14	9	9	7
S.aureus:	-	-	13	8	19	5	5	4
E.coli	-	-	14	6	17	8	8	7
P.aeruginosa	-	-	11	9	11	9	7	6

W: *Woodfordia fruticosa*; C: *Catharanthus roseus*; dash (-): no zone of inhibition

Here methanolic extracts were more effective than other solvent extracts in both the plants with (14mm/9mm, 19mm/4mm, 17mm/8 mm, 11mm/9mm) zone of inhibition followed by and chloroform extracts of *Woodfordia fruticosa*/*Catharanthus roseus* plant leaves which gave (13/7mm, 13/8mm, 14/6mm and 11/9mm) zone of inhibition against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa* respectively. The highest zone of inhibition obtained in the methanolic extracts of *Woodfordia fruticosa* against *Staphylococcus aureus* followed by *Escherichia coli* whereas lowest found in aqueous extracts against *Staphylococcus aureus* in both the plants.

DISCUSSIONS

The present study was done under the objectives of extraction, screening, TLC, Antioxidant test, quantification of Tannin, and antibacterial assay of *Woodfordia fruticosa* and *Catharanthus roseus*. On the basis of visual observation of different solvent extracts of *Woodfordia fruticosa* and *Catharanthus roseus* leaf showed that concentration of crude extracts was highest in methanolic extracts whereas lowest in hexane extracts in both plants as reported earlier by (Grover *et al*., 2014; Abegunde and Ayodele-Oduola, 2013). Initial qualitative test is valuable in the revealing natural bioactive compound which subsequently may show the way to drug discovery and improvement (Mallikarjuna *et al*., 2007) and also an increasing interest in the phytochemical compounds, which could be relevant to their nutritional incidence and their role in health and disease (Steinmetz *et al*., 1991). On the basis of active physical and chemical standards of drug and in order to evaluate the efficacy of drug herbal drug standardization is an important footstep. In addition, a number of different secondary metabolites is present in a plant extract but phytochemical analysis will disclose simply a low range of its constituents (Grover *et al*., 2014). The present study of phytochemical analysis for such an important metabolites revealed that both plants showed positive results for their presence in their respective solvents (table 1) and results also indicated that *Woodfordia fruticosa* and *Catharanthus roseus* leaves are rich source of these secondary metabolites, which have high medicinal importance. In the qualitative phytochemical screening using various solvent extracts of plant, it was found biologically active phytochemicals were present in the chloroform, methanolic and aqueous extracts of both plants leaves. In other words, the results confirmed the presence of therapeutically potent compounds in leaf extract of both plants leaves. It revealed that *Catharanthus roseus* has higher amount of alkaloids and terpenoids while *Woodfordia fruticosa* showed higher amount of tannins, saponins, cardiac glycosides and flavonoid as compared to each other in their respective effective solvents (table 1). The previous study by Grover *et al*., (2014); Dubey *et al*., (2014); Dubey and Sushma, (2014) and Kabesh *et al*., (2015) also showed the presence of these metabolites in *Woodfordia fruticosa* and *Catharanthus roseus* leaves extracts with different solvents by different methods. These results indicate that *Woodfordia fruticosa* plant is a good source of phenolics and other metabolites which support its use in most of the regions where people consume this herb as a herbal medicines for various purposes and may be used as alternative source for herbal medicines.

TLC Results of *Woodfordia fruticosa* and *Catharanthus roseus*

For the extraction of secondary metabolites from the plant extracts and its detection, chromatographic and spectroscopic techniques have proved themselves as very useful technique (Kataria *et al*., 2011). Due to the simplicity and cost benefit thin layer chromatography (TLC) has been widely used in herbal drug standardization process (Aman *et al*., 2008). TLC profiling confirms the number of plant extracts towards confirmation of various metabolites. Various bioactive compounds have diverse Rf values in various solvent extracts (Grover *et al*., 2014). Here in the present study *Woodfordia fruticosa* and *Catharanthus roseus* leaf extracts of effective solvents showed conformation of presence of these metabolites with reference. The RF value obtained is approximately similar for both the plants with only one spots for each metabolite (table 2). Similar results also observed by Grover *et al*., (2014) in case of *Woodfordia fruticosa* where they confirm the presence of tannin, flavonoid and other metabolites. Similarly Kabesh *et al*., (2015) also confirm the secondary metabolites present in methanol and aqueous extracts *Catharanthus roseus* leaves. This result indicates that both plants is a rich source of important secondary metabolites.

Determination of Antioxidant Capacity by Glutathione Assay

Herbal medicines derived from plant extracts are being used to treat a wide variety of clinical disease (Luper, 1999). Natural antioxidants has been given more attention due to protective effects against drug-induced toxicity studies especially whenever free radical generation is involved (Ramadose *et al*., 2012). Therefore several efforts have been made in order to find out antioxidant potential of medicinal plants which can neutralize free radicals and prevent harm to liver and other vital organs, thereby reducing stress. Hence a comparative study was done to evaluate antioxidant property of *Woodfordia fruticosa* and *Catharanthus roseus* leaf powder. Glutathione, a major non-protein thiol in living organisms, plays a central role in coordinating the body's antioxidant defense processes. Excessive peroxidation causes increased glutathione consumption. Reduced thiols have long been reported to be essential for recycling of antioxidants like vitamin E and vitamin C (Kayang, 2007).

In the present study of antioxidant test of *Woodfordia fruticosa* and *Catharanthus roseus* leaves extracts by Glutathione assay revealed that *Catharanthus roseus* leaf extracts has more antioxidant property as compare to *Woodfordia fruticosa* leaves extracts with value 25.09 µmole/mg and 1.14 µmole/mg respectively. Therefore we can say that *Catharanthus roseus* has more potential towards neutralizing free radicals as compare to *Woodfordia fruticosa* and more useful as anti-cancerous drug formulations. So the steps towards increasing the glutathione content from these plants should be developed. Previously Patharajan and BalaAbirami (2014) and Finos *et al*., (2011) revealed antioxidant property of *Catharanthus roseus* and *Woodfordia fruticosa* leaves by DPPH assay.

Quantification of Tannin

Tannins and tannin-like substances are widespread in nature and play important role in prevention of chronic diseases. It exerts anti-inflammatory, antimicrobial, antioxidant, anti-carcinogenic and body mass reducing activities (Saxena *et al*., 2013; Treas and Evans, 1992). Herbs that have tannins as their main component are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery (Subhuti, 2003). In the present study of quantitative estimation of tannin revealed that tannin percentage (w/w of tannic acid) in 1 gm sample of *Woodfordia fruticosa* leaf was higher (1.496 %) as compare to *Catharanthus roseus* (0.693 %). Previous reports of tannin quantification were also reported in *Woodfordia fruticosa* and *Catharanthus roseus* leaves by Khan *et al*., (2011); Rao and Ahmad, (2014). It

indicates that *Woodfordia fruticosa* is more astringent in nature and will be used as a better option for treating intestinal disorders such as diarrhea and dysentery as compare to The Tannins fraction have high medical activities thus steps need to be developed to *Catharanthus roseus*. Further steps to increase the tannin content of the plant and also good extraction steps for extracting tannins will be required.

Antibacterial Assay

The importance of *Woodfordia fruticosa* in the community can perhaps be understood by the broad spectrum of its antibacterial activity and in case of Catharanthus roseus has been mostly studied with respect to its anticancer, anti hypertension and anti diabetic properties and till date, very few efforts have been made to check the antimicrobial characteristics. In the present study of antimicrobial properties of *Woodfordia fruticosa* and *Catharanthus roseus* leaves extracts from different solvent showed that out of four solvent leaves extracts methanol, chloroform and water extracts of *Woodfordia fruticosa* and *Catharanthus roseus* extracts were effective against studied bacterial colonies. Hexane extracts of both the plants were ineffective against these bacteria. In all the solvents extracts *Woodfordia fruticosa* leaves extracts showed higher zone of inhibition than *Catharanthus roseus* leaf extracts against studied microbes. The methanolic extracts were more effective than other solvent extracts in both the plants. Methanolic extracts of *Woodfordia fruticosa*/*Catharanthus roseus* plant leaves gave higher inhibition zone (14mm/9mm, 19mm/4mm, 17mm/8 mm, 11mm/9mm) and chloroform extracts of *Woodfordia fruticosa*/*Catharanthus roseus* plant leaves gave (13/7mm, 13/8mm, 14/6mm and 11/9mm) zone of inhibition against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa* respectively. The present study of *Woodfordia fruticosa* showed agreement with the results of Bhattarai and Bhuju, 2011 and partial agreement with the dubey et al 2015 in which they found antibacterial activity in hexane extracts of *Woodfordia fruticosa* leaves and in case of *Catharanthus roseus* showed agreement with result reported by Kabesh *et al*., 2015. Antifungal activity also of *Catharanthus roseus* is reported by Kumari and Gupta, (2013). The present results of antimicrobial activity and its conformation with the previously reported work we can say that *Woodfordia fruticosa* and *Catharanthus roseus* leaf extract should be used as potent inhibitors of infectious bacterial organism and used as antimicrobial drug for infectious diseases. We hope that the results of antimicrobial activities may play a significant role in the conservation of traditional medicine knowledge of *Woodfordia fruticosa* and *Catharanthus roseus* and encourage the scientific community for further investigations of antimicrobial activity will be required to see the effectiveness of these extracts against other infectious microbial agents.

CONCLUSIONS

The results obtained in this present study conclude that *Woodfordia fruticosa* was more stringent on the basis of its high tannine concentration whereas *Catharanthus roseus* had more antioxidant power as compare to each other. Based on the antibacterial property methanolic extracts of *Woodfordia fruticosa*, it would be used as better option for future drugs discoveries to cure various diseases.

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